

Received: 2017.12.18
Accepted: 2018.01.08
Published: 2018.01.23

Does Use of Lidocaine Affect Culture of Synovial Fluid Obtained to Diagnose Periprosthetic Joint Infection (PJI)? An *In Vitro* Study

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 **Kan Liu**
BDF 2 **Liyan Ye**
BCF 3 **Wei Sun**
ADEG 1 **Libo Hao**
ADG 2 **Yanping Luo**
ADF 1 **Jiying Chen**

1 Department of Orthopedics, General Hospital of Chinese People's Liberation Army, Beijing, P.R. China
2 Department of Clinical Microbiology, General Hospital of Chinese People's Liberation Army, Beijing, P.R. China
3 Intensive Care Unit, Nanyuan Hospital, Beijing, P.R. China

Corresponding Author: Jiying Chen, e-mail: jiyingchen_301@163.com
Source of support: National Natural Science Foundation of China (NSFC) (grant no. 81672192)

Background: Synovial fluid culture (SFC) is recommended as one of the major diagnostic criteria by the Musculoskeletal Infection Society (MSIS) for diagnosing periprosthetic joint infection (PJI). Local anesthetic agents are used for anesthesia and analgesia in some clinical settings to relieve pain. As a local anesthetic, lidocaine is safely used in arthrocentesis to obtain synovial fluid. The goal of this study was to determine if infiltration anesthesia with additive-free lidocaine 2% has antibacterial effects that might interfere with subsequent SFC.


Material/Methods: Eight isolates of reference strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Streptococcus pyogenes*, and *Candida albicans* were incubated on the plates. Each bacterial suspension was formed by 50-fold dilution before the test lidocaine 2% was added. For each strain, bacterial suspension was divided into 2 groups (5 samples each) exposed either lidocaine 2% or sterile non-bacteriostatic 0.45% saline. The antimicrobial property of lidocaine 2% was determined by measuring the bacterial density on agar plates incubated for 24 h and comparing it with controls unexposed to lidocaine 2%.

Results: Exposure to lidocaine 2% negatively affected microbial viability *in vitro*. Of the lidocaine 2% exposure, reference strains but no *Streptococcus pyogenes* strain resulted in fewer colony-forming units compared with the sterile saline control. The antibacterial property of lidocaine 2% appears to affect the ability to culture the organism in synovial fluid.

Conclusions: Lidocaine 2% has strong antimicrobial activities against some commonly encountered bacterial strains in PJI. As a result, infiltration anesthesia with additive-free lidocaine 2% before the arthrocentesis procedure may affect the results of SFC. To further evaluate its potential antibacterial usefulness in clinical applications, studies are needed to assess the ability of lidocaine to reduce the risk of iatrogenic infections.

MeSH Keywords: **Anti-Bacterial Agents • Bacterial Infections • Colony Count, Microbial • Culture • Lidocaine • Synovial Fluid**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/908585>

 2165

 1

 1

 28



Background

Periprosthetic joint infection (PJI) is a catastrophic complication following total joint arthroplasty (TJA), which accounts for 15% of failed hip arthroplasties and 25% of failed knee arthroplasties [1]. However, the nonspecific symptoms and test results make the diagnosis of PJI quite challenging [2]. In the management of PJI, the ability to identify septic and aseptic failures of the prosthesis would be critical for the surgeon in deciding on optimal treatment of PJI [3]. Notably, isolation of bacteria from synovial fluid or periprosthetic tissue is essential to determine antimicrobial susceptibilities of the organisms. Furthermore, identifying bacteria could help individually prepare the antibiotic-loaded bone cement spacer for performing a 2-stage exchange arthroplasty [4–6].

The microbiological analysis of synovial fluid and periprosthetic tissue, the hematology, and imaging tests are important routines for diagnosing PJI, but some of these results are nonspecific and nonsensitive for PJI. To address the inconsistency of different tests for diagnosing PJI, the first guideline as a reference for the diagnosis of PJI was published in 2010 by the American Academy of Orthopedic Surgeons (AAOS), which states that joint aspiration should be performed when the ESR and CRP are elevated [7]. Moreover, in 2011, the Musculoskeletal Infection Society (MSIS) renewed this with a consensus statement providing a concise definition of PJI [8]. As one of the major diagnostic criteria in MSIS, the microbiological diagnosis of PJI performed by analyzing synovial fluid culture (SFC) or periprosthetic tissue culture is recommended. However, the definitive diagnosis of PJI remains difficult, and is mainly characterized by technical limitations and unsatisfactory results of culture.

Synovial fluid is commonly obtained by arthrocentesis, which is an essential medical procedure for the diagnosis and treatment of joint diseases [9]. However, the procedure may require local anesthesia to relieve pain. According to the results of earlier studies, several local anesthetics with various contents were found to have antibacterial activity [10–12]. However, their antibacterial effects only occur at higher doses [13]. Thus, their main effects are used instead of their antibacterial activity in daily clinical practice, especially in local application for anesthesia.

As a local anesthetic, lidocaine is already used before the procedure of arthrocentesis. Based on reports on the properties of lidocaine against some bacteria *in vivo* and *in vitro*, its use in microbiological sampling can pose a problem by causing false-negative results [14–17]. Based on the literature, we hypothesized that the results of SFC are adversely affected by use of lidocaine for local anesthesia. To date, there are no findings of whether lidocaine affects SFC in diagnosing PJI. Thus, we

designed an *in vitro* study to mimic the clinical situation with regard to isolates exposed to solutions of lidocaine 2%. The purpose of this study was to determine if lidocaine 2% interferes with the ability to culture the pathogenic bacteria commonly encountered in PJI.

Material and Methods

To preliminarily examine the concept that lidocaine can produce false-negative results of SFC, an *in vitro* experiment was performed to test the antibacterial properties of lidocaine 2% on common PJI pathogenic bacteria. The bacteria used in this study were 8 isolates of reference strains of *Staphylococcus aureus* (SAU) (ATCC 25923), *Staphylococcus epidermidis* (SEP) (ATCC 14990), *Staphylococcus hominis* (SHO) (ATCC 27844), *Escherichia coli* (ECO) (ATCC 11775), *Klebsiella pneumoniae* (KPN) (ATCC 13883), *Acinetobacter baumannii* (ABA) (ATCC 19606), *Streptococcus pyogenes* (SPY) (ATCC 12344), and *Candida albicans* (CAL) (ATCC 90028; all originally from the American Type Culture Collection, Manassas, VA). The experiment quantified the density of bacteria on agar plates. Colony-forming units (CFU) were counted and results are expressed as CFU/ml for samples.

We performed 10-fold, 25-fold, 50-fold, and 100-fold dilutions, and quantitatively cultured 10 µl of the resultant fluids by plating onto Mueller Hinton (MH) agar (bioMerieux) plates to estimate the density before the test lidocaine 2% was added. We prepared an optimal inoculum size of 3×10^6 colony-forming units (CFU)/ml. This was achieved by culturing the bacteria overnight on blood agar, China blue agar, and Sabouraud's agar plates at 35°C (*Streptococci* were cultured in a CO₂-rich atmosphere). All cultures were then diluted with sterile non-bacteriostatic 0.45% saline to achieve a 0.5 McFarland standard of approximately 1.5×10^8 CFU/ml, which were then diluted to reach a final concentration of approximately 3×10^6 CFU/ml. This was achieved by diluting 100 µl of the 0.5 McFarland standard with 4.9 ml of sterile saline by using a sterile pipette and its tip.

Quantitative culture was performed on each bacterial suspension to which lidocaine 2% was added. In addition, as a control group, a sample of each bacterial suspension was unexposed to lidocaine 2% and was plated onto MH agar (bioMerieux) and MH agar with 5% sheep blood (bioMerieux) (for SPY) plates for 24 h at 35°C (SPY in CO₂-rich atmosphere). The sample of each bacterial suspension in either exposed groups or unexposed groups was incubated on 5 agar plates.

The concentration of lidocaine was chosen to simulate clinical practice and circumstance in our center. Tests with lidocaine were prepared by modifying previously described methods [18,19]. Initially, 2 polystyrene tubes for each isolate were

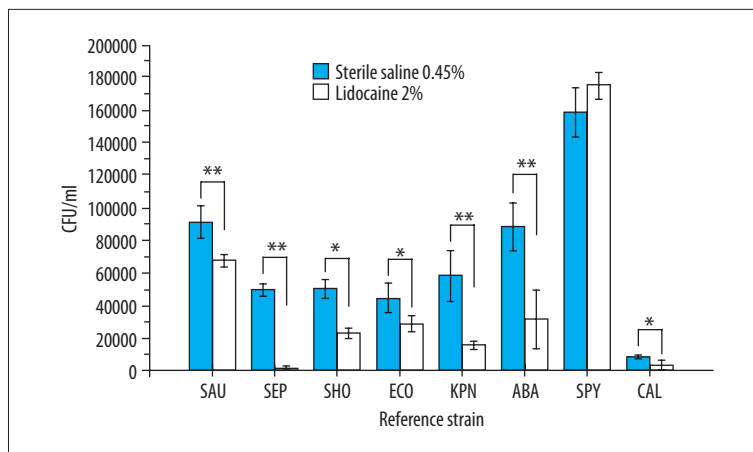


Figure 1. The mean density of bacteria incubated on agar plates was quantified after 24 h (and standard deviation). * $P<0.05$ and ** $P<0.01$ for the difference between lidocaine 2% and sterile saline 0.45% group as shown by the t test or the Mann-Whitney U test. CFU – Colony-forming unit, SAU – *Staphylococcus aureus*; SEP – *Staphylococcus epidermidis*; SHO – *Staphylococcus hominis*; ECO – *Escherichia coli*; KPN – *Klebsiella pneumoniae*; ABA – *Acinetobacter baumannii*; SPY – *Streptococcus pyogenes*; CAL – *Candida albicans*.

prepared, one containing 100 μ l lidocaine and 600 μ l bacterial suspension (3×10^6 CFU/ml), and the other containing 100 μ l sterile saline and 600 μ l bacterial suspension (3×10^6 CFU/ml). Then, the tubes were mixed for 1 min and the resultant suspensions of 10 μ l were inoculated onto MH agar plates and incubated for 24 h at 35°C (SPY in a CO₂-rich atmosphere). After incubating on the agar plates for 24 h, colonies were counted by 2 independent researchers.

Statistical analysis

SPSS 17.0 software (IBM, Chicago, IL) was used to compare data among groups. Data are presented as mean \pm standard deviation for continuous variables. These data included *in vitro* CFU counts of the lidocaine group and control group, compared using the t test or the Mann-Whitney U test. Values of $P<0.05$ were considered significant for all statistical comparisons.

Results

Quantitative culture of ATCC strains quantified the density of bacteria on agar plates. Exposure to lidocaine 2% decreased microbial viability except for the SPY strain. With regard to lidocaine 2%, a test with sterile saline as a growth control showed no antibacterial effect (Figure 1). Quantitative culture of SAU, SEP, SHO, ECO, KPN, ABA, SPY, and CAL exposed to lidocaine 2% showed an estimated mean bacterial density (\pm standard deviation) of $(6.74\pm 0.40)\times 10^4$, $(0.19\pm 0.08)\times 10^4$, $(2.29\pm 0.33)\times 10^4$, $(2.83\pm 0.51)\times 10^4$, $(1.57\pm 0.26)\times 10^4$, $(3.15\pm 1.80)\times 10^4$, $(17.5\pm 0.83)\times 10^4$, and $(0.32\pm 0.29)\times 10^4$ CFU/ml, respectively. Mean bacterial density on agar plates in the unexposed controls were $(9.11\pm 0.99)\times 10^4$, $(4.93\pm 0.37)\times 10^4$, $(5.02\pm 0.59)\times 10^4$, $(4.44\pm 0.90)\times 10^4$, $(5.79\pm 1.56)\times 10^4$, $(8.83\pm 1.52)\times 10^4$, $(15.84\pm 1.54)\times 10^4$, and $(0.86\pm 0.11)\times 10^4$ CFU/ml, respectively (Table 1).

When exposed to lidocaine 2%, there was a very rapid and significant reduction of all test strains except for SPY within 24 h compared with saline controls ($P<0.05$) (Figure 1). SAU, SEP, ABA, and KPN were the most susceptible bacteria with a significant reduction within 24 h ($P<0.01$). Inhibition of CAL, ECO, and SHO was also assessed at 24 h of exposure, with significant inhibition of these strains within 24 h ($P<0.05$). Only SPY was less susceptible when exposed to lidocaine 2% after 24 h, with no significant reduction shown on the plates ($P=0.1164$).

Discussion

Surgeons use local anesthetic agents like lidocaine for anesthesia and analgesia in some minor surgical interventions. Local anesthetic agents possess antimicrobial properties [20]. Earlier studies have demonstrated that the antibacterial effects of local anesthetic agents are related to changes in permeability of membrane properties of bacteria, inhibition of membrane-bound enzymes and protein RNA and DNA synthesis, alterations in characteristic ultrastructure, and lysis of protoplasts [13,21]. Results in the present study showed that from lidocaine 2% exposure, reference strains but no SPY strain resulted in lower numbers of colony-forming unit compared with the sterile saline control. We conclude that 2% lidocaine has strong antimicrobial activities against some commonly encountered bacterial strains in PJI.

Lidocaine is a local anesthetic agent with known antibacterial activity [19,22–24]. Miller et al. [14] reported that bacteriologic investigation of samples taken during dermal and subdermal minor surgical interventions can lead to false-negative results when lidocaine is used for these procedures. Stratford et al. [25] found a 70% decrease in bacterial density when lidocaine injected in infiltrative form was inoculated into the area. Based on these studies, our experiment also used lidocaine in liquid form, applied by infiltrative injection. Our purpose was not to show the possibility of using lidocaine as an antibiotic

Table 1. Bacterial density on agar plates in exposed groups and control groups* ($\times 10^4$ CFU/ml).

Reference strain	Lidocaine 2%	Sterile saline 0.45%	P value
SAU	6.74±0.40	9.11±0.99	<0.01 [#]
SEP	0.19±0.08	4.93±0.37	<0.01 [#]
SHO	2.29±0.33	5.02±0.59	<0.05 [#]
ECO	2.83±0.51	4.44±0.90	<0.05 [#]
KPN	1.57±0.26	5.79±1.56	<0.01 [#]
ABA	3.15±1.80	8.83±1.52	<0.01 [#]
SPY	17.5±0.83	15.84±1.54	0.1164
CAL	0.32±0.29	0.86±0.11	<0.05 [#]

* Data are mean \pm standard deviation. CFU – Colony-forming unit; SAU – *Staphylococcus aureus*; SEP – *Staphylococcus epidermidis*; SHO – *Staphylococcus hominis*; ECO – *Escherichia coli*; KPN – *Klebsiella pneumoniae*; ABA – *Acinetobacter baumannii*; SPY – *Streptococcus pyogenes*; CAL – *Candida albicans*. [#] Significant.

but, rather, to test whether it had any antimicrobial effects and provided false-negative results in SFC for diagnosing PJI.

With lidocaine 2% versus sterile saline controls, we found significant reduction of bacterial density on MH agar plates incubated at 35°C for 24 h, except for a single reference strain of SPY, which was not decreased after 24 h of incubation. The additive-free lidocaine 2% solution appears to have certain antimicrobial effects. Our results are consistent with an often-cited study by Schmidt et al. [15], who found an 80.1% inhibition of the 1219 clinical bacterial isolates from 28 different species when exposed to lidocaine 2% *in vitro* at 37°C after 18–24 h of incubation. Moreover, SAU was found to be totally resistant to lidocaine 1%, but we found a visible reduction of SAU in lidocaine 2% after 24 h. This difference may be due to the absence of culture medium and the low concentration of lidocaine. As in a study of the antibacterial properties of lidocaine, the minimal inhibitory concentration (MIC) of lidocaine toward any of the tested bacteria has been determined to not be below 0.5% [26]. In contrast to our study, they found SPY to be the most susceptible bacterium, with a total sensitivity to lidocaine 2%. In other studies, SPY was demonstrated to be susceptible in lidocaine 1%, with only a 2-h survival [19]. Similarly, the MIC of lidocaine in culture media was found to be 2% for SPY [27]. However, our results confirm that lidocaine 2% did not affect SPY, which was previously reported to be resistant to several local anesthetic agents and chemical disinfectants [12]. Again, culture media and culture conditions may have contributed to the differences.

As mentioned above, the results of bacterial reduction found at 37°C were almost the same as we found at 35°C; therefore, temperature does not seem to affect the antimicrobial property of lidocaine 2%. Lidocaine was shown to have concentration-dependent antibacterial activity in some *ex vivo*

experiments [13,23,28]. Nevertheless, the present study did not attempt to test whether antimicrobial effect varied with different concentrations, so a single concentration of lidocaine used routinely in our department was chosen in order to mimic clinical practice of synovial fluid sampling. Lidocaine, a well-known local anesthetic for local infiltration anesthesia in many conditions, such as arthrocentesis, percutaneous tissue biopsy, and minor surgical interventions, may have antimicrobial activity with the use of its routine concentration, as in our study. Thus, lidocaine 2% may be inappropriate for the preparation of synovial fluid sampling, even though the additive-free solution is only used to anesthetize the skin, subcutaneous tissues, and capsule, and very little if any is injected into the joint. In addition, the procedures of infiltration anesthesia and synovial fluid sampling are also performed in sequence without changing the needle, so lidocaine residue in the needle tubing can become mixed into the synovial fluid used for culturing. Therefore, the warnings of antibacterial activity stated in lidocaine must be taken into consideration, especially in the preparation of synovial fluid sampling. From the aforementioned findings, we also hypothesized the antibacterial property of lidocaine 2% may have a positive side-effect in reducing the potential risk of septic arthritis posed by tissue coring with epidermis and dermis into the joint during arthrocentesis. However, whether lidocaine 2% helps to prevent such an iatrogenic infection caused by breaking the integrity of the dermis remains to be investigated in further clinical studies.

The present study is limited by using a single local anesthetic with a single concentration, lidocaine 2%, at a level consistent with clinical use. Based on our literature review, there is no study on the antibacterial effects of lidocaine and SFC. Because the solution is used in local infiltration anesthesia, we added a sample of the additive-free lidocaine 2% to the tube

containing bacterial suspension; therefore, the form and concentration of lidocaine used in this study were very close to our clinical usage. Thus, we do not think this limitation renders our results unreasonable.

Conclusions

The colonies of viable bacteria measured for lidocaine 2% indicate the antibacterial property of this agent *in vitro*. As the bacterial density incubated in this study was supra-physiological, we have good reason to believe that lidocaine 2% has

strong antibacterial effects against the common PJI pathogenic bacteria in clinical applications. Therefore, we conclude that infiltration with additive-free lidocaine 2% before synovial fluid sampling for culture may be a potential factor affecting the results of SFC. On the other hand, for it to be considered for the preparation of other clinical interventions like joint injection, further investigations are needed to demonstrate whether lidocaine reduces the risk of iatrogenic infection.

Acknowledgments

We thank the staff who were involved in this study.

References:

- Bozic KJ, Kurtz SM, Lau E et al: The epidemiology of revision total knee arthroplasty in the United States. *Clin Orthop Relat Res*, 2010; 468: 45–51
- Del Pozo JL, Patel R: Clinical practice. Infection associated with prosthetic joints. *N Engl J Med*, 2009; 361: 787–94
- Tande AJ, Patel R: Prosthetic joint infection. *Clin Microbiol Rev*, 2014; 27(2): 302–45
- Garvin KL, Konigsberg BS: Infection following total knee arthroplasty: Prevention and management. *J Bone Joint Surg Am*, 2011; 93: 1167–75
- Trampuz A, Piper KE, Jacobson MJ et al: Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med*, 2007; 357(7): 654–63
- Mariconda M, Ascione T, Balato G et al: Sonication of antibiotic-loaded cement spacers in a two-stage revision protocol for infected joint arthroplasty. *BMC Musculoskelet Disord*, 2013; 14: 193
- Parvizi J, Della Valle CJ: AAOS Clinical Practice Guideline: Diagnosis and treatment of periprosthetic joint infections of the hip and knee. *J Am Acad Orthop Surg*, 2010; 18(12): 771–72
- Parvizi J, Zmistowski B, Berbari EF et al: New definition for periprosthetic joint infection: From the Workgroup of the Musculoskeletal Infection Society. *Clin Orthop Relat Res*, 2011; 469(11): 2992–94
- Xu C, Peng H, Chai W et al: Inadvertent introduction of tissue coring into joints during arthrocentesis: an experimental study. *Med Sci Monit*, 2017; 23: 3571–77
- Maddi R, Horrow JC, Mark JB et al: Evaluation of a new cutaneous topical anesthesia preparation. *Reg Anesth*, 1990; 15: 109–12
- Vanscheidt W, Sadjadi Z, Lillieborg S: EMLA anaesthetic ointment for sharp leg ulcer debridement: A review of the clinical evidence for analgesic efficacy and tolerability. *Eur J Dermatol*, 2001; 11: 90–96
- Johnson SM, Saint John BE, Dine AP: Local anesthetics as antimicrobial agents: A review. *Surg Infect (Larchmt)*, 2008; 9(2): 205–13
- Sedef Gocmen J, Buyukkocak U, Caglayan O, Aksoy A: *In vitro* antibacterial effects of topical local anesthetics. *J Dermatolog Treat*, 2008; 19(6): 351–53
- Miller MA, Shelley WB: Antibacterial properties of lidocaine on bacteria isolated from dermal lesions. *Arch Dermatol*, 1985; 121: 1157–59
- Schmidt RM, Rosenkranz HS: Antimicrobial activity of local anesthetics: Lidocaine and procaine. *J Infect Dis*, 1970; 121: 597–607
- Wimberley N, Willey S, Sullivan N, Bartlett JG: Antibacterial properties of lidocaine. *Chest*, 1979; 76: 37–40
- Gocmen JS, Buyukkocak U, Caglayan O: *In vitro* antibacterial activity of some systemic and topical antihistaminic preparations. *Clin Invest Med*, 2009; 32(6): E232
- Schweitzer ME, Deely DM, Beavis K, Gannon F: Does the use of lidocaine affect the culture of percutaneous bone biopsy specimens obtained to diagnose osteomyelitis? An *in vitro* and *in vivo* study. *Am J Roentgenol*, 1995; 164(5): 1201–3
- Berg JO, Mössner BK, Skov MN et al: Antibacterial properties of EMLA and lidocaine in wound tissue biopsies for culturing. *Wound Repair Regen*, 2006; 14(5): 581–85
- Tustin A, Kim SJ, Chomsky A et al: Antibacterial properties of 2% lidocaine and reduced rate of endophthalmitis after intravitreal injection. *Retina*, 2014; 34(5): 935–42
- Silva MT, Sousa JCF, Polonia JJ, Macedo PM: Effects of local anesthetics on bacterial cells. *J Bacteriol*, 1979; 137: 461–68
- Olsen KMT, Peddicord E, Campbell GD, Rupp ME: Antimicrobial effects of lidocaine in bronchoalveolar lavage fluid. *J Antimicrob Chemother*, 2000; 45: 217–19
- Parr AM, Zoutman DE, Davidson JS: Antimicrobial activity of lidocaine against bacteria associated with nosocomial wound infection. *Ann Plast Surg*, 1999; 43: 239–45
- Kerenyi M, Batai MR, Juhasz V, Batai I: Lidocaine/prilocaine ointment (EMLA) has an antibacterial effect *in vitro*. *J Hosp Infect*, 2004; 56: 75–76
- Stratford AF, Zoutman DE, Davidson JS: Effect of lidocaine and epinephrine on *Staphylococcus aureus* in a guinea pig model of surgical wound infection. *Plast Reconstr Surg*, 2002; 110: 1275–79
- Craig SB, Concannon MJ, McDonald GA, Puckett CL: The antibacterial effects of tumescent liposuction fluid. *Plast Reconstr Surg*, 1999; 103: 666–70
- Zaidi S, Healy TE: A comparison of the antibacterial properties of six local analgesic agents. *Anaesthesia*, 1977; 32: 67–70
- Aydin ON, Eyigor M, Aydin N: Antimicrobial activity of ropivacaine and other local anaesthetics. *Eur J Anaesthesiol*, 2001; 18: 687–94